Kunio MITUI*, Narumi NAKATO** & Shigeo MASUYAMA***:

Studies on intraspecific polyploids of the fern *Lepisorus thunbergianus* (2)**** Cytogeography of main cytotypes

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ノキシノブの種内倍数体の研究 (2) 主要サイトタイプの分布

In the first paper of this series (Nakato et al. 1983), we reported the distribution patterns of the seven cytotypes (2n=50, 51, 75, 76, 100, 101 and 102) of *L. thunbergianus* in Kanto districts. The main cytotypes in this area were the diploid with 2n=50, the tetraploid with 2n=100, and the hypertetraploid with 2n=102. From the fact that diploids are predominant in coastal and lowland regions, while tetraploids and hypertetraploids in inland and upland regions, it was suggested that the diploids are distributed in relatively warm climatic regions, while the tetraploids and the hypertetraploids in rather cold climatic regions. In this study, a cytogeographical survey of these three cytotypes has been extended to other regions of Japan in order to confirm this trend substantially. In the survey, spore morphologies obtained from the herbarium specimens as well as the chromosome numbers of living materials have been employed as one of the useful criteria for the identication of ploidy.

Materials and methods Meiotic chromosome counts were made with the usual aceto-carmine squash technique, and mitotic counts were made with the same manner as described in the previous paper (Nakato et al. 1983). Spores were collected from fresh plants authenticated cytologically and the herbarium specimens of TNS. For the measurement of spore sizes, they were mounted in Canada balsam and fifty spores were measured in every specimen. For the section, mature spores were embeded in 1% agar, fixed in the FAA fluid, and dehydrated in the following series; 30%, 50%, 70%, 85%, 95% and 100% ethanol and GMA (glycol methacrylate) mixture (1:1). These specimens were embedded in GMA monomer mixture, polymerized at 60°C for 3 hours, and sectioned by 2 microns with a glass knife. The aqueous solution of Giemsa was employed for the staining of the sections. For scanning electron microscopy, spores were mounted on specimen stubs with white cemedine, and coated with gold. Microphotographs were made at 10 KV accelerating voltage with Hitachi-S-800 scanning electron microscopy.

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Results Chromosome counts. Chromosome numbers were determined for the plants collected from the following localities (numbers of plant materials are given in parentheses):

Diploid (2n=50): Isl. Miyakejima (2), Isl. Hachijojima (1), Tokyo Pref.; Ito (2), Yugashima (2), Heda (2), Shizuoka Pref.; Kaifu (1), Tokushima Pref.; Kumamoto City (1), Kumamoto Pref.; Isl. Yakushima (1), Kagoshima Pref.; Isl. Iriomotejima (1), Okinawa Pref.

Tetraploid (2n=100): Akita City (1), Akita Pref.; Ohtaba (2), Itsukaichi (2), Tokyo Pref.; Daisen (1), Tottori Pref.

Hypertetraploid (2n=101): Ikawa (1), Akita Pref.

Hypertetraploid (2n=102): Juniko (6), Aomori Pref.; Ikawa (5), Akita City (5), Akita Pref.; Yahiko (3), Wajima (1), Niigata Pref.

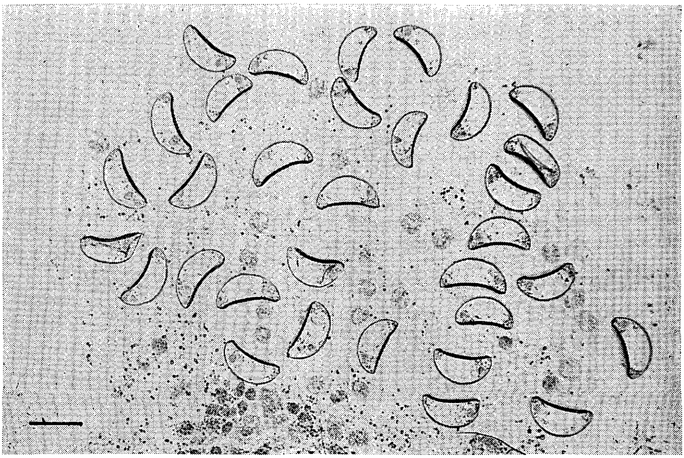

Fig. 1. 32 spores/sporangium observed in the materials at Ohtaba, Tokyo Pref. Scale shows 50 μm.

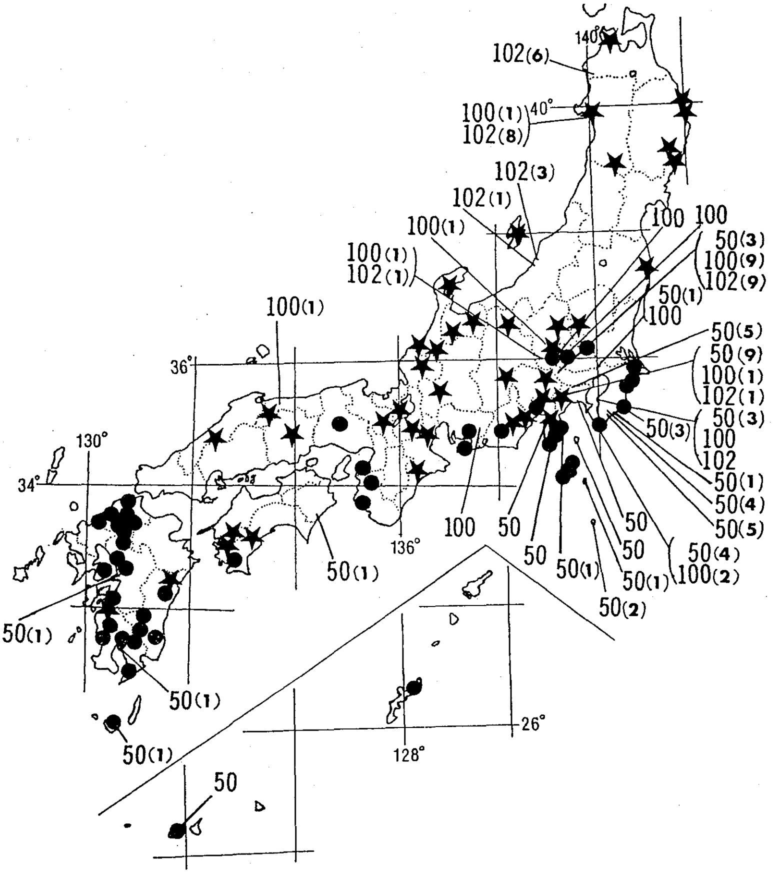

Fig. 2. Distributions of three main cytotypes (2n=50, 100 and 102) in the Japanese Archipelago. The numbers show the somatic chromosome number. In parentheses the number of specimens used for the measurements of spore size are given. The localities of herbarium specimens are represented by solid circles (diploid type) and asterisks (tetraploid type).

Hypertetraploid (2n=103): Ikawa (1), Akita Pref.

The last cytotype with 2n=103 was newly found in this study. It is interesting to note that a sporangium bearing 32 spores instead of 64 normal ones observed in the course of chromosome observation with tetraploid materials collected at Ohtaba, Tokyo Pref. (Fig. 1). Geographical distributions of three main cytotypes, 2n=50, 100 and 102, are shown in Fig. 2.

Spore observations. Data for the spore size in cytological voucher specimens are given in Fig. 3. As seen in this figure, there is no appreciable difference in the mean spore size between tetraploid and hypertetraploid plants. However, the mean spore sizes, especially the mean equatorial diameters of spores, of diploid plants are considerably smaller than those of tetraploid and hypertetraploid plants, though a little overlap exists between them. The mean spore size was 59.4±2.6 μm SD (equatorial diameter) × 37.8±1.8 μm SD (polar diameter) in 46 diploid plants, 67.8±3.7 × 41.3±3.0 μm in 16 tetraploids and 67.2 ±2.4 × 41.4±1.9 μm in 29 hypertetraploids. As calculated on the basis of these standard deviations, it seems possible to use 63 μm in the mean equatorial diameter as an indicative value to distinguish diploid plants from tetraploid and hypertetraploid plants to a considerable degree. Actually of 46 diploid materials examined, 43 plants (93%) show less than 63 μm in the mean equatorial diameter, and 42 (93%) of 45 tetraploid and hypertetraploid plants show more than 63 μm.

As for spore ornamentation, the reticulate pattern formed by the projections of the exospore was observed on spores. Although the perispore was not found under the optical microscope, thin membraneous perispore were sometimes ob-

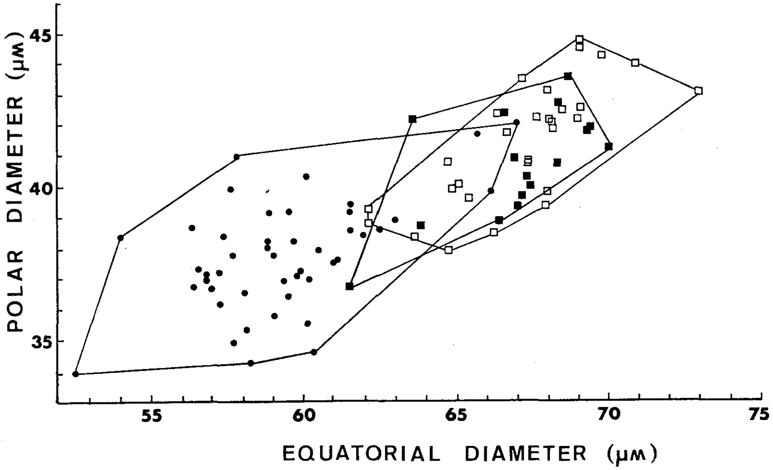

Fig. 3. Scatter diagram showing the mean spore sizes in the cytological voucher specimens. Solid circle, diploid (2n=50); solid square, tetraploid (2n=100); open square, hypertetraploid (2n=102).

served on the surface of the exospore by the SEM. The thicking of the exospore is partial in the spores of diploid plants. However it extends to the whole exospore in the spores of tetraploid and hypertetraploid plants. Therefore, the muri are fatter, higher and lesser per unit area, and the lumina are larger in the spores of diploids, as compared with the spores of tetraploids and hypertetraploids (Figs. 4 & 5). Such differences of the sculpture were also used for the determination of the ploidy level of herbarium specimens. The herbarium specimens whose ploidy level has been settled through spore observations are as follows:

Diploid type: Sashima-gun (TNS 412480), Ibaraki Pref.; Chichibu (402484), Hiki-gun (293383), Saitama Pref.; Katori-gun (385909), Togane (404161), Sosa-gun (405743), Ohara (405723), Tateyama (323254), Chiba Pref.; Isl. Niijima (415861), Isl. Shikinejima (415863), Isl. Kozujima (415865), Tokyo Pref.; Ito (414548), Kawazu (33230), Shimoda (312255, 412464), Fuji-gun (414566), Haibara-gun (414562), Shizuoka Pref.; Nukata-gun (387179), Atsumi (412305), Aichi Pref.; Sennan-gun (248656), Osaka Pref.; Higashimuro-gun (416744), Gobo (416722), Ito-gun (416748), Wakayama Pref.; Asago-gun (410302), Hyogo Pref.; Sukumo (414305), Kochi Pref.; Chikujo-gun (399251), Kurate-gun (399254, 399260), Fukuoka (399232, 399233), Amagi (399224), Yame (399241), Omuta (399248), Kitakyushu (399263), Chikushi-gun (399223), Nogata (399259), Fukuoka Pref.; Tamana (410965), Minamata (410944), Kumamoto Pref.; Shimabara (406129), Nagasaki Pref.; Kitamorokata-gun (417588), Minaminaka-gun (418068), Higashiusuki-gun (418086), Nishimorokata-gun (418058), Miyazaki Pref.; Hioki-gun (401069, 401092, 401129), Kimotsuki-gun (418086), Isl. Yakushima (401126), Aira-gun (401110), Satsuma-gun (401070), Soo-gun (401061), Kagoshima Pref.; Isl. Okinawa (402520), Isl. Iriomotejima (402507), Okinawa Pref.

Tetra- or hypertetraploid type: Kitatsugaru-gun (TNS 409366), Aomori Pref.; Akita (418587), Ogachi-gun (418591), Akita Pref.; Kunohe-gun (404022), Kuji (404023), Kesen-gun (404032), Ohfunato (404026), Iwate Pref.; Futaba-gun (401607), Fukushima Pref.; Sado (402763), Niigata Pref.; Aso-gun (331179), Tochigi Prof.; Tano-gun (401451), Seta-gun (412536), Gunma Pref.; Ashigarakami-gun (405652), Kanagawa Pref.; Otsuki (415692), Yamanashi Pref.; Kaminiikawa-gun (402561) Higashitonami-gun (395885), Toyama Pref.; Nagano (420751), Kamiina-gun (420739), Nagano Pref.; Fujinomiya (414568), Numazu (414527), Tagata-gun (414580), Haibara-gun (414583), Sunto-gun 410934), Shizu-

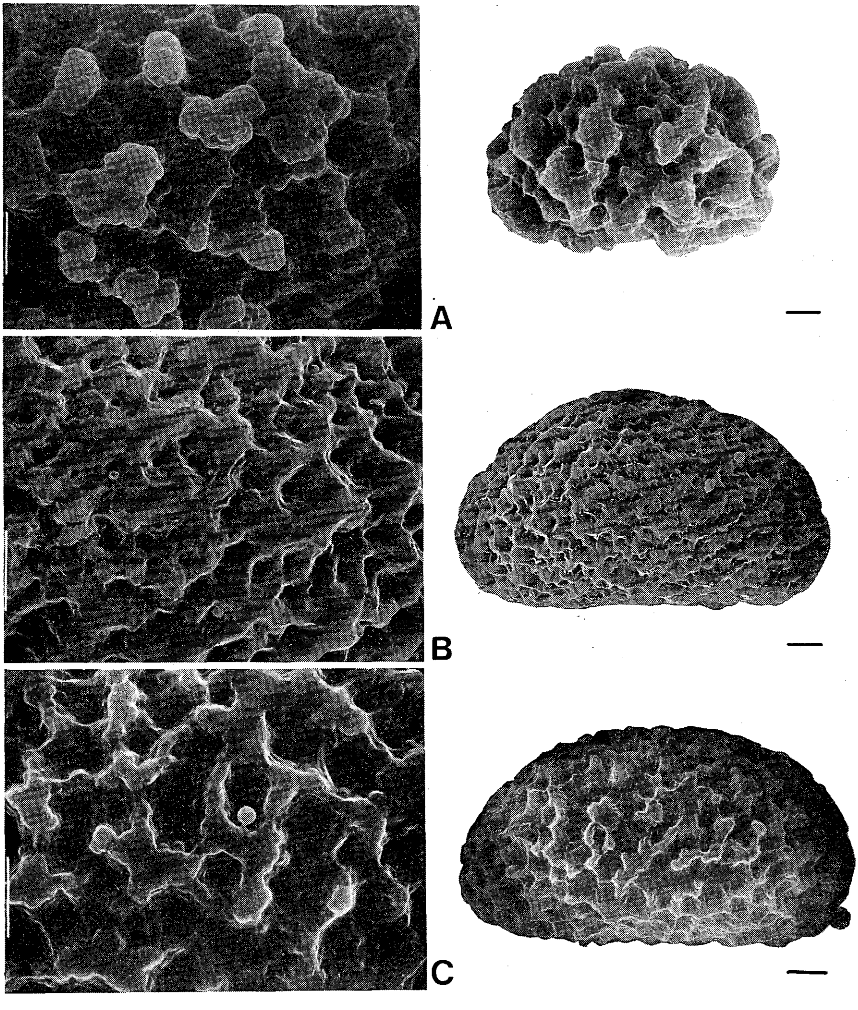

Fig. 4. Microphotographs of spores (right) and surfaces of exospores (left) in three cytotypes of *L. thunbergianus*. A: Diploid ($2n=50$, Katsuura, Chiba Pref.), B: Tetraploid ($2n=100$, Kamakura, Kanagawa Pref.), C: Hypertetraploid ($2n=102$, Juniko, Aomori Pref.). Scales show 5 μm.

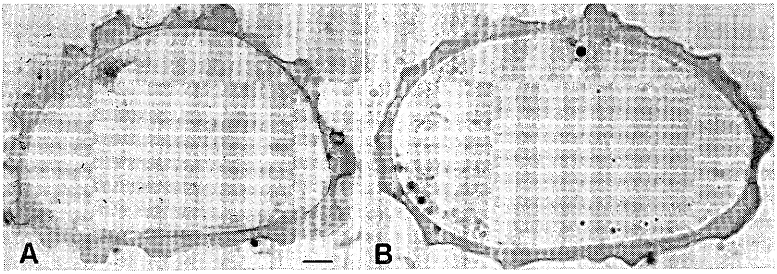

Fig. 5. Sections of spore wall in the diploid (A) and tetraploid (B) of *L. thunbergianus*. Scale shows 5 μm.

oka Pref.; Motosu-gun (222568), Gifu Pref.; Ishikawa-gun (272494), Fugeshi-gun (411562), Enuma-gun (411570), Ishikawa Pref.; Eiheiji (406537), Fukui Pref.; Suzuka-gun (s. n.), Taki-gun (406301), Mie Pref.; Kanzaki-gun (409123), Takashima-gun (409114), Kaga-gun (409128), Shiga Pref.; Kyoto (395867), Kyoto Pref.; Hino-gun (401620), Tottori Pref.; Kume-gun (399161), Okayama Pref.; Ochi-gun (398461), Iishi-gun (398457), Shimane Pref.; Takaoka-gun (414329), Kochi Pref.; Kitauwa-gun (282163), Higashiuwa-gun (413805), Ehime Pref.; Usuki-gun (417496), Miyazaki Pref.; Izumi (401076), Kagoshima Pref.

The localities of these herbarium specimens are shown in Fig. 2

Discussion From Fig. 2, it is obvious that the diploid and the tetraploid group ($2n=100$ and 102) are different considerably in distribution patterns through the Japanese Archipelago. The diploid cytotype is restricted in southern half of Japan, from Okinawa to Kanto districts, while the tetraploid group is widespread over Japan, extending from Kyushu to Tohoku districts. It is now evident from this feature that the diploids favor rather warm climatic regions, while the tetra- and hypertetraploids rather cold climatic regions, as suggested in the previous study (Nakato et al. 1983). In this connection, it is worthy to note that in the northern distribution range of this species, i. e. Aomori, Akita and Niigata Prefs., 20 plants (87%) of 23 materials examined cytologically were of hypertetraploid with $2n=102$. The high occurence frequency of hypertetraploids in northern districts indicates that the hypertetraploids are more adapted for northern cold climate, as compared with the tetraploids.

Cytogeographical surveys through the Japanese Archipelago have been made for several fern species, and the similar situation to that of *L. thunbergianus* has been reported for the following species : diploids and tetraploids in *Woodwardia orientatis* (Mitui 1968), diploids and triploids in *Dryopteris erythrosora* (Hirabayayashi 1974), diploids and tetraploids in *Pteris dispar* (Nakato 1981). In these species, ancestral diploid cytotype occurs in southern regions and derived polyploidal cytotypes in northern regions. However, in any of these cases, there is no or a little overlapping area of distribution between different cytotypes, so that a demarcation line is recognizable between distribution ranges of them. On the contrary, in *L. thunbergianus* the distribution ranges of the diploid and the tetraploid group overlap in several regions, as reported by Mitui (1968), Takei (1974, 1978) and Nakato et al. (1983). In relation to this feature, two observations should be noted. Mitui (1968) observed a duplicated chromosomes with $n=52$ (4x number) in meiosis of a diploid plant ($n=26$) of *L. thunbergianus* var. *angustus*, which grew in more inland and upland than var. *thunbergianus*. In the present study, some sporangia containing 32 spores instead of normal 64 ones, which indicated chromosome doubling in spore mother cells, were observed in two tetraploid materials from Ohtaba, Tokyo Pref. Considering these observations and widely overlapped distributions of the diploids and the tetraploids of this species, it seems that tetraploids have recently or even currently been originated directly from diploids by the unreduced spore formation with duplicated chromosomes which may enable them to get a wider tolerance for a cold climate.

As to the origin of hypertetraploids, it is plausible from the chromosome numbers that the hypertetraploids with $2n=102$ are of hybrid origin between the diploids with $2n=50$ and a variety of this species, var. *angustus* with $2n=52$. On the other hand, in the studies on karyotypes and nuclear DNA contents, Takei (1982) reported that the tetraploids and the hypertetraploids of *L. thunbergianus* may be of hybrid origin between the diploid cytotype of *L. thunbergianus* and *L. onoei* ($2n=50$). However, it is still possible to consider that the tetraploids of this species originated directly from the diploids in terms of the chromosome doubling, and/or the hypertetraploids originated through the hybridization between the diploids and the variety of this species var. *angustus*, as suggested above.

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References

Hirabayashi, H. 1974. Cytogeographic studies on *Dryopteris* of Japan, Hara Shobo, Tokyo. Mitui, K. 1965. Journn. Jap. Bot. **40**: 117-124. —— 1966. ibid. **41**: 270-276. —— 1968. Sci. Rep. Tokyo Kyoiku Daigaku, Sec. B. **13**: 285-333. —— 1971. Journ. Jap. Bot. **46**: 83-93. —— 1975. Bull. Nippon Dental Coll., Gener. Educ. No. 4, 221-271. Nakato, N. 1981. Journ. Jap. Bot. **56**: 200-205. ——, S. Masuyama & K. Mitui 1983. ibid. **58**: 195-205. Takei, M. 1974. Journ. Jap. Bot. **49**: 356-359. —— 1978. ibid. **53**: 87-91. —— 1982. Res. Bull. Fac. Educ., Oita Univ. **6**: 9-41.

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第1報でノキシノブの種内倍数体の関東地方における分布を調査し，主要なサイトタイプは2倍体 (2n=50)，4倍体 (2n=100)，高4倍体 (2n=102) であり，2倍体は温暖域に，4倍体と高4倍体はより寒冷域に分布することを報告した。今回，これらのサイトタイプの日本における分布を調査した。生材料の染色体観察のみでなく，胞子の大きさや表面模様が2倍体と4倍体群で異なるので，胞子の形質を使用して腊葉標本の倍数性も推定し，分布図を得た。その結果，2倍体は沖縄～関東地方，4倍体群は九州～東北地方に分布することが判明した。水平分布では重なりがみられるが，2倍体は温暖地域に，4倍体群は寒冷地域に分布することを確認した。また，分布の北限に近い東北地方では，染色体観察により，高4倍体が数多く分布していることがわかった。これは，高4倍体は4倍体よりもより寒冷な気候に適応していることを示している。また，4倍体群の起源に関して，2倍体の胞子形成時の染色体倍加によって，2倍体から直接4倍体が形成される可能性があること，さらにノキシノブ2倍体とナガオノキシノブ2倍体 (2n=52) の雑種がもとになって高4倍体が生じる可能性があることを指摘した。

□鄭英昊ほか10名：**韓国植物分類学史概説** 404pp. 1986. 図書出版, Seoul. 10,000Won. 管束植物，淡水藻類，淡水珪藻類，海洋植物プランクトン，海藻類，陸水学に分けてそれぞれの研究史が記述されている。管束植物の部分が約半分を占める。それぞれの章に詳細な文献リスト（管束植物では45頁約1000件）がついているので，韓国の植物研究を概観するのに有用である。全文韓語。 (金井弘夫)